BIOPHYSICAL PROPERTIES OF BACTERIOPHAGE T2

DONALD J. CUMMINGS* AND LLOYD M. KOZLOFF

Committee on Biophysics and Department of Biochemistry, University of Chicago, Chicago, Ill. (U.S.A.)

(Received February 23rd, 1960)

SUMMARY

Biophysical properties of bacteriophage T2 and T2 "ghosts" have been studied using the ultracentrifuge, the electron microscope and the diffusion apparatus. The molecular weight of T2 at either pH 7 or pH 5 has been found to be $215 \cdot 10^6$. The protein portion of the head of the intact particles or of the DNA-free phage ghost has been found to exist in two forms, depending on both pH and temperature. One head form of intact T2 is approximately 15 % longer than the other; 119 mμ by 80 mμ *vs.* 103 × 80 mμ. Raising either the temperature or the pH favors the formation of the longer head form. This change in head form accounts for the dual sedimentation phenomenon observed now for T2 ghosts and reported previously for intact T2. Estimates have been made of the thermodynamic changes involved in the transition of one form to the other.

INTRODUCTION

HOOK, BEARD, TAYLOR, SHARP AND BEARD reported in 1946 that T2 bacteriophage particles exhibit the unusual property of having two different sedimentation coefficients depending upon the suspending medium[1,2]. The sedimentation coefficient was about 700 S** in 0.9 % NaCl and about 1000 S in 0.023 *M* $CaCl_2$. The appearance of these two forms was also dependent on the pH of the solvent, at a pH greater than 5.8, the 700 S (slow) form existed, while below pH 5.8, only the 1000 S (fast form) was observed. BENDET, SWABY AND LAUFFER[3] later demonstrated that the transition of the fast to the slow form took place in less than 2 min.

Two explanations for this dual sedimentation behavior of T2 have been proposed. SHARP *et al.*[2] suggested that orientation of specifically aggregated particles could result in two such sedimentation coefficients. However, PUTNAM presented evidence indicating that the particles were not oriented during sedimentation[4]. This conclusion was challenged[3] on the grounds that the particles could have been oriented already at field strengths lower than PUTNAM employed. It was argued that orientation of

Abbreviations: PTA, phosphotungstic acid, DNA, deoxyribonucleic acid.

* Present address: University Institute of Microbiology, Copenhagen, Denmark. Pre-doctoral Fellow of U.S. Public Health Service.

** All sedimentation coefficients will be given in Svedberg units (10^{-13} sec) (S) and will be corrected, when possible, to a water basis at 20°. The sedimentation coefficient in pH 7 buffer will sometimes be referred to as S_7 and in pH 5 buffer as S_5. Similar notations will be used for the diffusion coefficients at these pH values.

these unusually shaped viruses could be brought about by the density heterogeneity due to the DNA inside the viral head. Later TAYLOR, EPSTEIN AND LAUFFER[5] and BENDET *et al.*[3] measured the diffusion coefficients of T2 in pH 5 and pH 7 buffers, calculated the molecular weights of the two forms, and concluded that since both forms have similar molecular weights, the dual sedimentation phenomenon could not be due to aggregation. It seemed more likely that the difference in sedimentation properties was due to a change in the individual particles. BENDET *et al.*[3,6] then postulated that a reversible change in the shape of the phage particle occurred at the tip of the tail. Tail fibers were thought to be unwound or extended in the slow form thus retarding sedimentation while in the fast form the tail fibers were thought to be tightly wound and not to affect sedimentation. These views were based on what were considered discrepancies in the ratios of the diffusion coefficients to the sedimentation coefficients of the two forms and on the appearance of the virus in electron micrographs.

We have reexamined the diffusion properties of T2 phage and found no discrepancy between the sedimentation and diffusion constants. In view of the fact that the head of this particle contains over 90% of the mass of the particle, it seemed possible that a change in the physical properties, such as a 42% change in the sedimentation coefficient, would involve a change in the head structure. We have now found that the two sedimenting forms have different head lengths; the slower sedimentation form has a head length 13–16 mμ longer than the faster sedimenting form. It has also been found that the interconversion of the two forms is markedly affected by temperature.

EXPERIMENTAL

Materials

Stocks of T2r$^+$ were grown on *E. coli* B in liquid culture and purified and assayed by standard procedures[7]. Protein coats or "ghosts" of T2r$^+$ were prepared and purified by the osmotic shock method described by HERRIOTT AND BARLOW[8]. Whole phage and "ghosts" were stored in the cold in 0.9% NaCl, containing 0.001 *M* $MgSO_4$. Under the proper conditions, these preparations all gave single peaks in the ultracentrifuge. The phosphate content of samples of these preparations was found to be 3.9% by weight. This agrees well with the values of COHEN AND ANDERSON[9] for T2, SCHLESINGER[10] for WLL (presumably T2) and KOZLOFF AND PUTNAM[11] for T6, but is lower than the 5.0 to 5.2% reported by TAYLOR[12] and HERRIOTT AND BARLOW[13] for T2.

Diffusion rates

The diffusion coefficients of the fast and slow forms of whole T2 were measured in the Spinco Model H apparatus taking into consideration the effects of both pH and temperature. Since, as will be shown later, lowering the temperature of the suspending buffer tends to convert the slow form into the fast form, the pH of the buffer used in measuring the diffusion coefficient of the slower form must be high enough so that only the slow form exists at the relatively low temperature of the diffusion bath. Whole T2 at a concentration of $3 \cdot 10^{12}$/ml was suspended in sodium phosphate buffer, ionic strength 0.1, at a pH of 5.78 or 7.85 and the bath was kept at 7.1°. The diffusion coefficients were measured by the Rayleigh fringe method[14] and corrected to a water

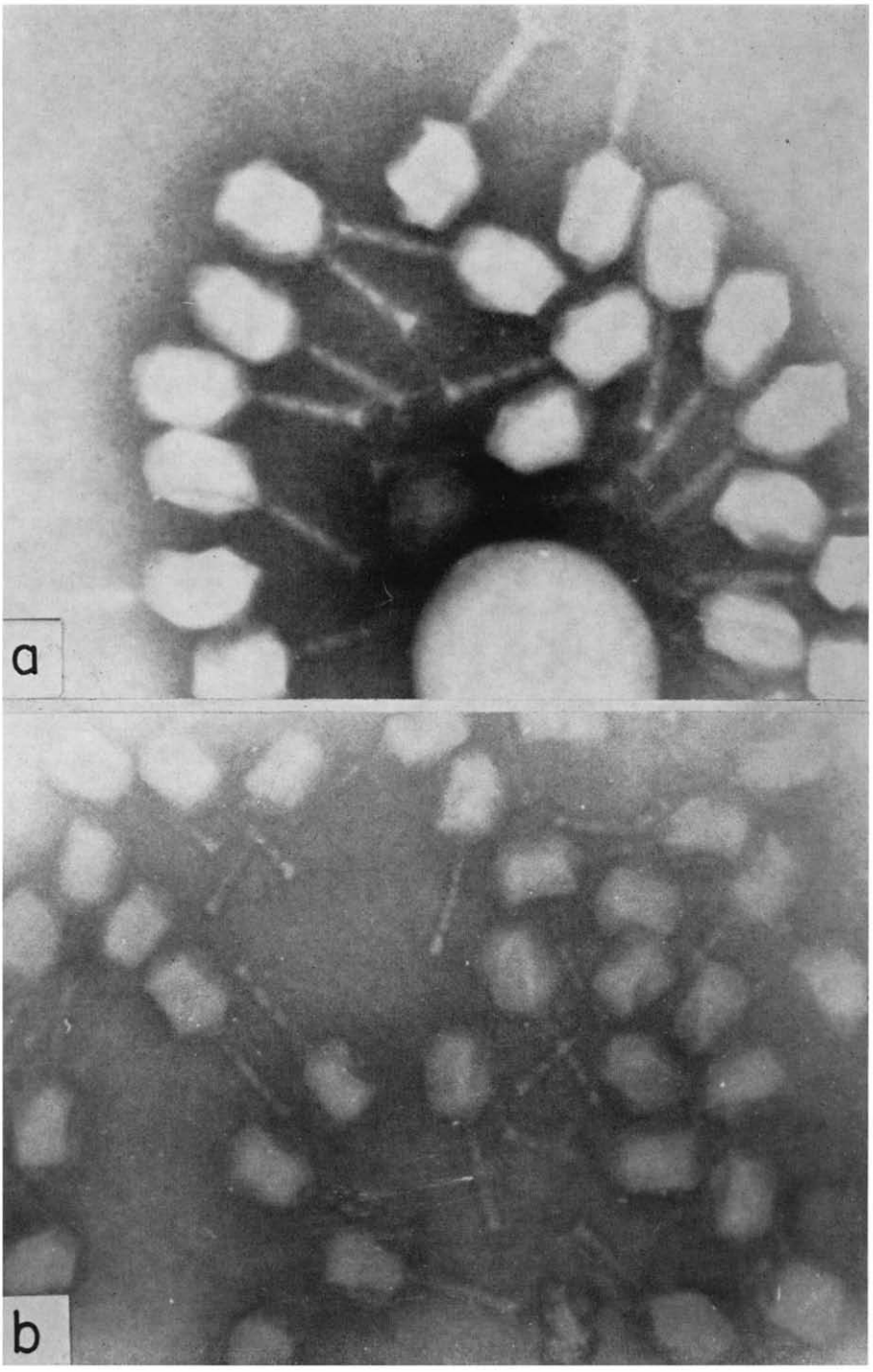

Fig. 1. Electron micrographs of the two forms of bacteriophage T2 using the PTA procedure after formol fixation. The magnification is 125,000 × for both pictures. (a) T2 at pH 7.0 and (b) T2 at pH 5.7.

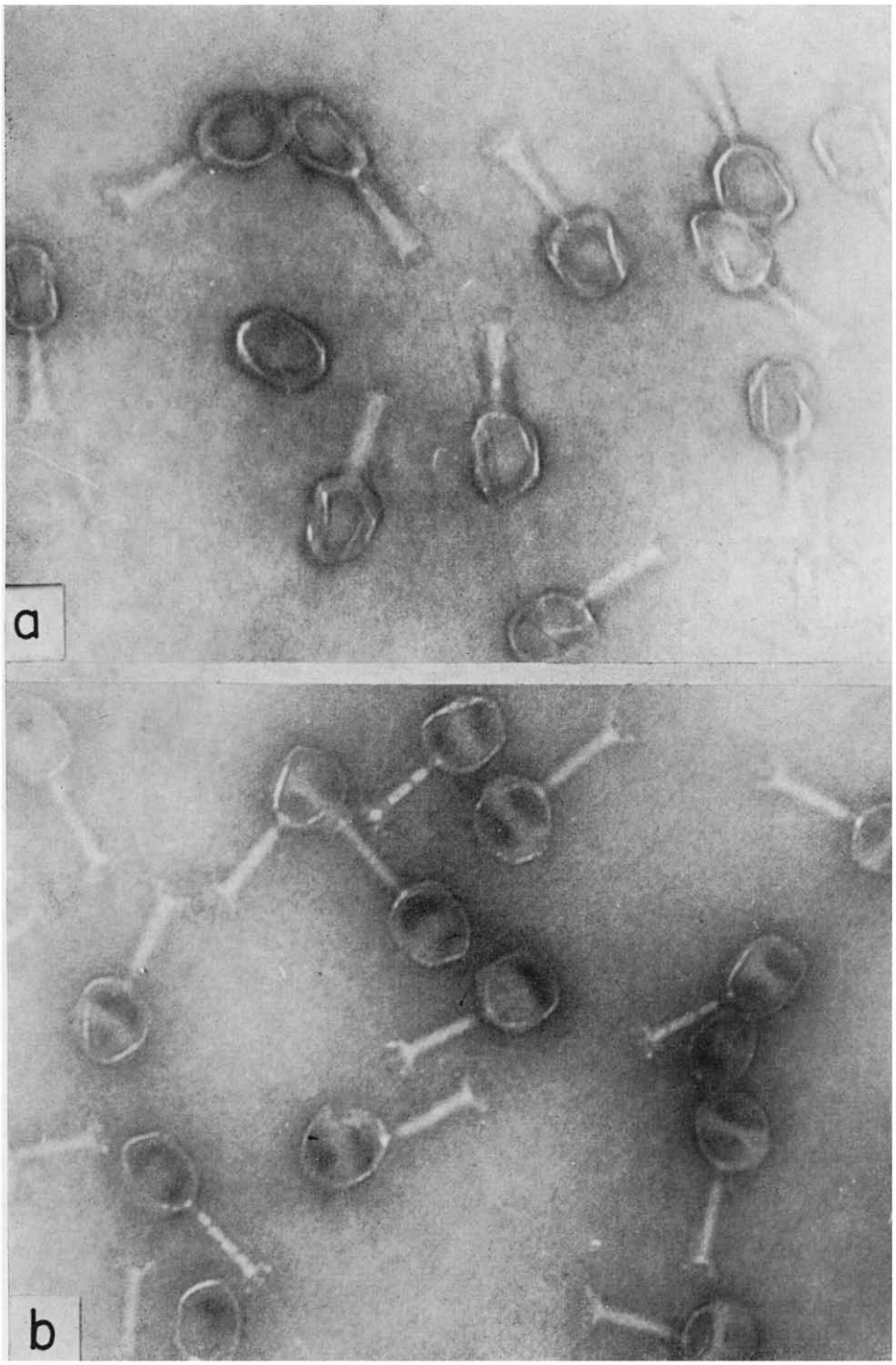

Fig. 2. Electron micrographs of the two forms of T2 "Ghosts" using the PTA procedure after formol fixation. The magnification is 100,000 × for both pictures. (a) T2 "ghosts" at pH 7.0; and (b) T2 ghosts at pH 5.7.

basis at 20°. The $D_{20,w}$ value of the form at pH 5.78 was found to be $3.40 \pm 0.10 \cdot 10^{-8}$ cm²/sec and that of the form at pH 7.85 to be $2.35 \pm 0.09 \cdot 10^{-8}$ cm²/sec. The ratio of $D5/D7$, 1.45 ± 0.07, calculated from these values is in good agreement with the ratio of 1.43 ± 0.05 obtained for $S5/S7$ (see later). Using the Svedberg formula and a partial specific volume of 0.66 ml/g (see ref. 12), the molecular weight of the fast form was found to be $214 \cdot 10^6$ and the slow form, $216 \cdot 10^6$. These data firmly place the cause of the dual sedimentation phenomenon of T2 on a change in individual particles

Morphological appearance of two phage forms

The respective sedimentation forms of whole T2 and T2 "ghosts" were fixed by the 2% formol treatment of BENDET, ALLISON AND LAUFFER[6] and examined in an RCA EMU-3 electron microscope. Both palladium shadowing and the PTA embedding method described by BRENNER AND HORNE[15] were used to increase contrast. Both methods showed that the slow form of whole T2 had a relatively longer head than the fast form. The axial ratio (length/width) of the head of over 200 individual particles in 4 separate experiments at pH 7.0 was 1.49 ± 0.07, while the axial ratio of the head of the form at pH 5.7 was 1.29 ± 0.05.

The dimensions of the head of the phage form after formol fixation in pH 7.0 buffer at 37° were 119 ± 4 mμ long by 80 ± 2 mμ wide. The face in the body of the head was 72 mμ long and apex height was 23 mμ. After fixation at pH 5.7 at 25°, the head was 103 ± 3 mμ long and was still 80 ± 2 mμ wide. The approximately 15 mμ shortening appeared to occur almost exclusively in the body of the head; the face of the short form was 58 mμ long as compared to 72 mμ in the longer form. No change was apparent in the height of the apices. Representative electron micrographs of these two forms of whole T2 are presented in Fig. 1.

Electron micrographs of the two forms which T2 ghosts (see later for sedimentation properties) assume are shown in Fig. 2. The head of the slower sedimenting form still largely retains the polyhedral shape of the phage particle although it deforms readily. The head has an axial ratio of 1.42 ± 0.05 and is 123 ± 5 mμ long by $85 \pm$ mμ wide. The faster sedimenting form has a rounder head with an axial ratio of 1.22 ± 0.13 mμ although there is some variation in the size of the particles. The heads are 106 ± 10 mμ long by 86 ± 6 mμ wide. One important difference between the two forms is that the fast form has a band (indicating a higher concentration of protein in the phosphotungstic procedure) at least 13 mμ wide across the center of the head and normal to the tail.

The tails of all intact phage particles as well as phage ghosts have identical configurations. The fibers at the tail tips are extended in all forms. This can be readily seen in Fig. 2 of the ghosts. The tails are 125 mμ long when measured using either the phosphotungstic acid method or palladium shadowing after formol fixation. The tail width, however, was 20 mμ after metal shadowing but only 13 mμ in the phosphotungstic preparation.

These dimensions for the form of T2 at pH 7.0 are 25% larger than those reported by WILLIAMS AND FRASER[16] for metal shadowed frozen-dried T2, head 95×65 mμ and tail 100 mμ long. It can be calculated that the size of frozen-dried T2 corresponds to the expected water-free virus particles while the particle measured after formol fixation corresponds to the form which is known to contain 0.6 gram of H_2O/g of virus[5]. For example, the volume of T2 after freeze-drying is $2.55 \cdot 10^{-16}$ cm³ and since

dry T2 has a density of 1.5, this corresponds to a weight of $3.8 \cdot 10^{-16}$ g. This value is in good agreement with the particle dry-weight calculated from the diffusion and sedimentation coefficients of $3.6 \cdot 10^{-16}$ g *i.e.*, $215 \times 10^6/6 \times 10^{23}$. The volume of formol-fixed T2 is $4.5 \cdot 10^{-16}$ cm^3 and using the hydrated density of 1.26 the net weight of a T2 particle would be 5.7×10^{-16} *g*. This value, based on the dimensions in the electron micrographs, is in excellent agreement with that calculated from the physical measurements and the degree of hydration of $5.8 \cdot 10^{-16}$ g [3.6×10^{-16} $(1 + .6) = 5.8\ 10^{-16}$ g].

Sedimentation analysis of whole T2

Analysis of the transition between the two sedimentation forms of T2 was performed in the Spinco Model E Ultracentrifuge. Virus concentrations of $2 \cdot 10^{12}$/ml were dialyzed for two days in sodium phosphate buffer, ionic strength 0.1, in the pH range of 5.78 to 7.54. At each pH studied, the temperature of the centrifuge rotor was varied over the range of 8 to 45°. It was found that the transition between the two forms of T2 was markedly dependent upon the temperature as well as the pH. At a particular pH, the fast form was transformed into the slow form as the temperature of the solution was increased. The $S_{20,w}$, temperature, and pH values are listed in Table I. The complete transition between the two forms occurred over a temperature range of about 13° at a given pH. Representative sedimentation diagrams are presented in Fig. 3, showing the effect of temperature at pH 7.00. The average sedimentation coefficient of the slow form was 710 ± 25 S and that of the fast form, 1017 ± 11 S. The ratio of S5/S7, 1.43 ± 0.05, is in agreement with the value of 1.42 reported by BENDET *et al.*[3].

The effect of temperature on the sedimentation rate of whole T2 in NaCl and $CaCl_2$ solvents of 0.1 ionic strength was also examined. Each of these solvents had a pH of about 6.3. As seen in Table II, temperature also had a decided effect on the

TABLE I

SEDIMENTATION RATES OF WHOLE T2 BACTERIOPHAGE IN PHOSPHATE BUFFER

pH 5.78		*pH 6.06*		*pH 6.25*		*pH 6.58*	
Temperature	$S_{20,w}$	*Temperature*	$S_{20,w}$	*Temperature*	$S_{20,w}$	*Temperature*	$S_{20,w}$
9.3	1024	8.0	1022	10.0	1028	8.0	1032
16.1	1020	14.7	1027	15.5	1022	16.2	1009
20.1	1007	22.0	1031	20.9	1012	21.7	1008, 757
29.9	1000	29.8	962	28.9	952	28.8	714
37.6	970	37.1	794	35.8	702	35.7	681
45.0	672	44.2	661	42.4	650		

pH 7.00		*pH 7.34*		*pH 7.54*	
Temperature	$S_{20,w}$	*Temperature*	$S_{20\ w}$	*Temperature*	$S_{20,w}$
8.3	1021	8.3	1027, 757	9.8	738
11.7	1000, 737	12.5	1014, 748	14.1	731
18.3	1000, 745	16.4	1021, 729	19.7	699
23.9	704	21.9	698	24.6	686
30.6	674	28.9	662	28.9	670

Temperatures in ° C.

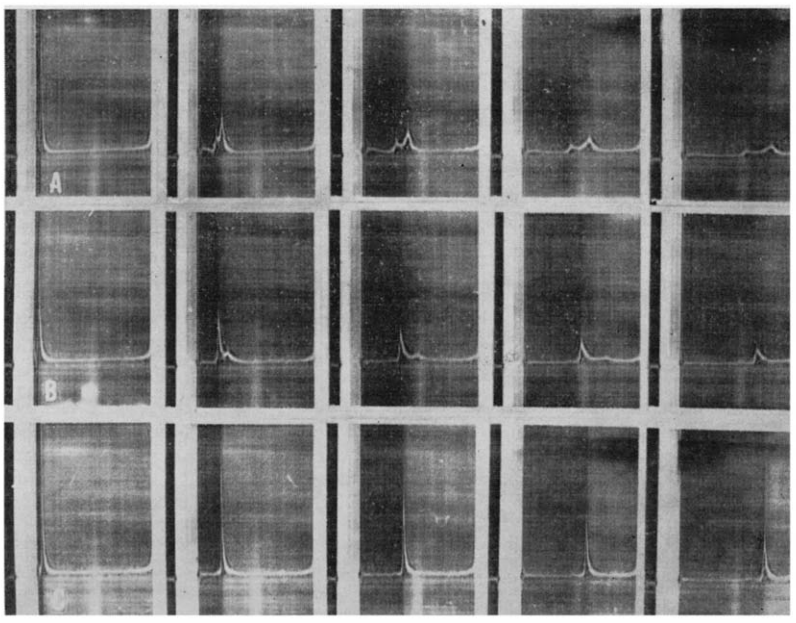

Fig. 3. Representative sedimentation diagrams of whole T2 showing the effect of temperature: (a) 15°, 747 S and 1016 S; (b) 22°, 742 S and 1063 S; and (c) 27°, 701 S.

TABLE II

SEDIMENTATION RATES OF WHOLE T2 BACTERIOPHAGE IN NaCl AND $CaCl_2$

In both cases the ionic strength was 0.1 and the pH was 6.3.

NaCl		*$CaCl_2$*	
Temperature °C	$S_{20,w}$	*Temperature* °C	$S_{20,w}$
19	1031	21	1094
25	1042, 773	27	1077
32	747	35	1086
40	704	42	1108

sedimentation rate of whole T2 in NaCl. This effect was similar to that obtained in sodium phosphate buffer. On the other hand, the presence of Ca^{++} appeared to keep the phage in the fast form even at temperatures where the phage in NaCl and sodium phosphate buffer was entirely in the slow form.

Sedimentation analysis of T2 "ghosts"

The sedimentation behavior of the protein coats of T2 was also studied as a function of pH and temperature. T2 "ghosts" at a concentration of $6 \cdot 10^{12}$/ml, were dialyzed for two days in sodium phosphate buffer, ionic strength, 0.1, in the pH range of 5.73 to 7.00. It was observed that T2 "ghosts" exhibit a dual sedimentation phenomenon similar to that of whole phage. The interconversion of the two forms of "ghosts" was markedly dependent upon the temperature as well as the pH of the solvent. The results are listed in Table III. From the data presented, it is evident

that intermediate forms can exist. At values of pH and temperature where both forms are present, the sedimentation rate consistently approaches the limiting value obtained at the pH where only one form exists. This effect was not observed with whole T2. The limiting values of the sedimentation coefficients of the two forms of T2 "ghosts" were taken as 256 ± 1S and 166 ± 1S; the ratio of $S5/S7$ is then 1.54 ± 0.01.

TABLE III

SEDIMENTATION RATES OF T2 "GHOSTS" IN PHOSPHATE BUFFER

pH 5.73		*pH 5.79*		*pH 5.86*	
Temperature	$S_{20,w}$	*Temperature*	$S_{20,w}$	*Temperature*	$S_{20,w}$
12.6	257	14.6	250	15.1	248, 184
18.0	255	20.0	251, 188	20.0	248, 180
24.0	256	23.2	249, 181	24.1	177
		27.3	181	28.5	176
		32.3	180	33.4	176
pH 5.92		*pH 5.99*		*pH 6.21*	
Temperature	$S_{20,w}$	*Temperature*	$S_{20,w}$	*Temperature*	$S_{20,w}$
9.2	247, 181	9.8	241, 170	9.2	221, 164
13.2	251, 182	16.2	247, 175	15.8	226, 167
18.0	246, 178	21.4	171	21.7	167
22.0	175	27.4	172	27.2	167
29.0	174	33.0	171	32.8	166

Temperatures in ° C.

Kinetic analysis of the interconversion of the two viral forms

The thermodynamic changes occurring in individual virus particles were calculated from the relative concentrations of the two sedimentation forms at various temperatures. Using these data and invoking a general reaction of the type

$$S7 + nX \rightleftharpoons S5 \tag{1}$$

where n is the number of moles of X/mole of T2 required for the transition, one can evaluate the $\Delta F°$, $\Delta H°$ and $\Delta S°$ values for the transition. Choosing the activating agent, X, of the transition was complicated by the fact that the transition was affected by both the buffer and the concentration of NaCl. Throughout this investigation and those of others previously mentioned, positively charged ions, particularly H^+ were observed to be directly concerned with the dual sedimentation phenomenon. For this reason, the role of H^+ was evaluated in detail and less attention was paid to Na^+ which also may have some effect.

As can be seen from equation (1), the equilibrium constant K is given by

$$K = \frac{(S5)}{(S7)\,(X)^n} \tag{2}$$

or,

$$-\log X = \frac{1}{n} \log \frac{(S7)}{(S5)} + \frac{1}{n} \log K \tag{3}$$

This is the equation of a straight line with a slope of $1/n$ and a y-intercept of $1/n \log K$. Eqn. (3) was utilized in obtaining values of n at different temperatures. Since the

sedimentation peaks were quite sharp, the relative concentration of the two forms was easily determined from the peak heights and the temperature, T^*, at which equal concentrations occurred, could be estimated to about $\pm$ 1°. The data in Table IV for T2 "ghosts" illustrates the calculation; and values of n for whole T2 were obtained in a similar manner. (If the activating agent was assumed to be Na^+ instead of H^+

TABLE IV

EVALUATION OF THE NUMBER OF MOLES OF H^+ INVOLVED IN THE TRANSITION OF T2 GHOSTS

Temperature °C	*pH*	S_7/S_5	*n* Moles of H^+
9.4	6.21	6/1	3.5
	5.99	6/7	
	5.22	2/3	
20	5.92	4/1	5.6
	5.86	8/5	
	5.79	8/11	

TABLE V

TEMPERATURE AT WHICH CONCENTRATIONS OF THE TWO FORMS OF T2 ARE EQUAL

The ionic strength was 0.1 in all experiments.

Whole T2			*T2 "Ghosts"*		
Solvent	*pH*	*Temperature* °C	*Solvent*	*pH*	*Temperature* °C
Phosphate	5.78	42	Phosphate	5.73	28
	6.06	37		5.79	22
	6.25	31		5.86	18
	6.58	24		5.92	15
	7.00	18		5.99	10
	7.34	9			
NaCl	6.3	25			
$CaCl_2$	6.3	>49			

values of n about ten-fold larger were obtained.) Values for n at other temperatures were obtained by assuming a linear relationship between temperature and n. In order to incorporate as much of the data as possible, the temperatures chosen for calculating $\Delta F°$, $\Delta H°$, and $\Delta S°$ were those at which equal concentrations of S_7 and S_5 existed. These temperatures are given in Table V. $\Delta H°$ was determined, using absolute temperatures T^*, from the VAN 'T HOFF equation,

$$\frac{\mathrm{d} \ln K}{\mathrm{d}T} = \frac{\Delta H^0}{RT^2}$$

If $\Delta H°$ is independent of temperature, a straight line relationship between $\log K$ and $1/T^*$ will result; this line will have a slope given by $-\Delta H°/2.303R$, from which $\Delta H°$ can be easily obtained. As seen in Fig. 4, a plot of $\log K$ *versus* $1/T^*$ yields the

desired straight line for whole T2 and T2 "ghosts" using H^+ (or Na^+) as the activating agent in the transition. The other variables $\Delta F°$ and $\Delta S°$ were calculated utilizing the relations,

$$\Delta F^0 = -RT \ln K \tag{5}$$

and

$$\Delta S^0 = \frac{\Delta H^0 - \Delta F^0}{T} \tag{6}$$

A comparison of the thermodynamic values at 22° is given in Table VI. It can be seen from Tables V and VI that the slow to fast form transition is qualitatively the same for whole T2 and T2 "ghosts". However, the transition of the "ghosts" occurs over a much narrower pH range and involves much larger changes in the thermodynamic variables. It must be emphasized that this is a real difference and is not due to merely a difference in concentrations of whole phage and "ghosts" examined in the ultracentrifuge. The magnitude of $\Delta F°$, $\Delta H°$ and $\Delta S°$ is not surprising because we are dealing with a reaction involving the entire molecular aggregate composing the head of T2 and not a simple molecular reaction.

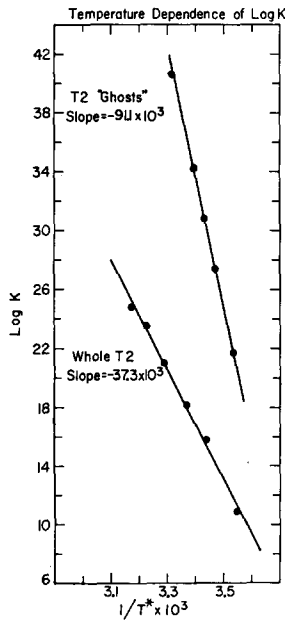

Fig. 4. The relationship between K, the equilibrium constant for the interconversion of the two forms of whole T2 or of T2 "ghosts", and $1/T^*$, the reciprocal of the absolute temperature at which the two forms exist in equal concentration.

TABLE VI

THERMODYNAMIC VALUES FOR THE SLOW TO FAST TRANSITION AT 22°

	Activating agent	*Δ F° kcal/mole*	*Δ H° kcal/mole*	*Δ S° e.u.*	*n (H⁺)*
T2 "ghosts"	H^+	—47.0	417	1573	6.0
Whole T2	H^+	—23.4	171	659	2.6

DISCUSSION

It is clear that the major morphological change involved in the dual sedimentation phenomenon of bacteriophage T2 is a variation in the dimensions of the head structure. Electron micrographs obtained by two different procedures show that the slow form has a 15% longer head than the fast form; this change in length occurs mainly in the body and immeasurably in the apices of the head. No other difference in appearance of the two forms was noted. That this variation in length of the head is due to a configurational change occurring in the head protein is demonstrated by the fact that protein coats or "ghosts" of T2 also exhibit two sedimentation coefficients. The main difference between whole phage and "ghosts" is that the latter has no DNA inside the protein coat. If the DNA were completely ineffective in the transition between the two forms, the estimates of $\Delta F°$, $\Delta H°$ and $\Delta S°$, and n, the number of activating protons, obtained would be the same with or without the DNA. The fact that they are not suggests that while the protein coat alone can undergo a change in length, DNA has some auxiliary action. This is also indicated by the intermediate values of $S_{20,w}$ obtained for T2 "ghosts". Apparently, when DNA is inside the phage head, the transition from one form to the other goes as an all-or-none reaction; when the DNA is removed, the dimensions of the head can change gradually until an unstable state is reached and the head then shifts rapidly to its limiting length.

The bonds being broken in the configurational change in the head structure must be weak, since the two forms are readily interchanged[3]. This implies that there must be a small change in heat content and entropy per bond broken and formed. The high values of $\Delta H°$ and $\Delta S°$ calculated for T2 "ghosts" indicate that a great many of these non-covalent bonds are being broken. While a large number of weak bonds are involved in the transition, the data indicated that relatively few positively charged ions are required to initiate the reactions and that probably the reaction is completed by some cooperative action of the protein subunits composing the viral head.

The main arguments for rejecting the contention of BENDET *et al.*[3,6] that the anomalous sedimentation and diffusion behavior of T2 was due to a difference in the configuration of the tail fibers are as follows: (a) Using procedures indentical to those of BENDET *et al.*[6], no difference in tail fiber configuration was observed, but changes in head shape were apparent. Moreover, the extended tail fibers shown by BENDET and his colleagues have not been observed after formol fixation by HERRIOT AND BARLOW[13] or by KELLENBERGER AND ARBER[17]. (b) The sedimentation properties of T2 "ghosts" are not in accord with BENDET's hypothesis. Removal of the DNA eliminates the density heterogeneity of these particles and greatly weakens any assumption of preferred orientation. Further, if the tail fiber model were correct, the number of activating ions required for the conversion of the slow to fast forms should be the same for whole T2 and "ghosts"; in contrast to this expectation, "ghosts" required 2 to 3 times as many activating ions as whole phage. (c) The tail fiber model was conceived to account for the apparent discrepancy in the ratios of $D5/D7$ and $S5/S7$. As shown in this paper, this discrepancy does not exist. LAUFFER's group[3,5] did not appreciate the effect of temperature on the transition between the two forms. At the pH and temperature used in their experiments, the phage could not have been maintained in the slow form. Hence, the ratio of $D5/D7$, 1.24, calculated from their results is in error. This effect of temperature also explains the different

values for the transition limits of pH reported in the literature. SHARP *et al.*[2] observed that above pH 5.8, only the slow form existed. However, LESLEY, FRENCH AND GRAHAM [18] obtained the fast form at pH 6.05. SINGER AND SIEGAL[19] found that the fast form existed at pH values as high as 6.9. In the course of their studies, BENDET *et al.*[3] indicated that the interconversion of the fast and slow forms of whole T2 covered about one-half a pH unit, with a central value at about pH 6.1. These apparent discrepancies in the effect of pH disappear when the role of temperature is considered.

When an attempt is made to account quantitatively for the differences in the diffusion and sedimentation coefficients of the two forms of T2 due to the change in head length, the importance of the change in the effective volume, V_e, is soon realized. The usual practice in relating the diffusion coefficient of a particle to its hydrodynamic shape is to use the EINSTEIN-SUTHERLAND equation, $D = kT/f$, where f is the frictional coefficient. According to ONCLEY[20], the frictional coefficient is a function of both the geometric dimensions and the effective volume, V_e. SCHERAGA AND MANDELKERN[21] modified the view that the effective volume is solely a function of the anhydrous partial specific volume and hydration by pointing out that this view neglects possible flow of solvent through the particle domain, deviation of the shape of the domain from that of an ellipsoid of revolution and deformation of the domain. With these modifications in mind, the frictional coefficient is,

$$f = 6\pi\eta\left(\frac{3V_e}{4\pi}\right)^{1/3}\left(\frac{f}{f_0}\right)_{\text{geom.}} \tag{7}$$

The shape dependent frictional coefficient can be calculated by assuming that the particle in question may be approximated by an equivalent ellipsoid of revolution. Using the equation derived by GANS[22] for randomly oriented prolate ellipsoids,

$$f_{\text{geom.}} = \frac{12\pi\eta\sqrt{b^2 - a^2}}{\ln\dfrac{b + \sqrt{b^2 - a^2}}{b - \sqrt{b^2 - a^2}}} \tag{8}$$

the shape dependent frictional coefficients of the fast and slow forms of T2 were calculated. The ratio $(f7/f5)_{\text{geom.}}$ was found to be 1.08. Assuming that the effective volumes of the two forms are unknown, their ratio may be determined from the ratio of $S5/S7$, 1.43 ± 0.05, obtained experimentally.

$$\frac{S_5}{S_7} = \frac{f7}{f5} = \left(\frac{V_{e7}}{V_{e5}}\right)^{1/3}\left(\frac{f7}{f5}\right)_{\text{geom.}} \tag{9}$$

The ratio of the effective volumes of the slow and fast forms, $Ve7/Ve5$, calculated in this manner was 2.3 ± 0.3. On the other hand, the ratio of the volumes determined from the dimensions of the two forms was found to be 1.24. This discrepancy may be partly due to the fact that the shape dependent frictional coefficient for ellipsoids is relatively insensitive to changes in the axial ratio. This inherent property tends to minimize the value of $(f7/f5)_{\text{geom.}}$ which in turn exaggerates the value of $Ve7/Ve5$ obtained from eqn. (9). Whether a tadpole-shaped object such as T2 phage can be approximated by an equivalent ellipsoid of revolution is itself debatable.

As suggested above, the problem of solvent flow through the particle, which could affect the hydroxynamic behavior of T2, must be considered. Two lines of evidence

point towards the conclusion that the two head forms of T2 behave differently with respect to solvent flow. ANDERSON, RAPPAPORT AND MUSCATINE[23] found that the head of the closely related bacteriophage T6 could exist in two osmotic shock forms. At a temperature of 42° and higher, this phage was found to be in a form resistant to osmotic shock. This finding correlates well with our results on the effect of temperature on the sedimentation properties of T2; it is highly probable that the osmotic shock resistant form is the slower sedimenting, long head form. Since susceptibility to osmotic shock is a function of the relative differences in permeability between solute and water, the long head form is more permeable to the flow of solvent than the short head, osmotic shock sensitive, form. In the studies on the sedimentation properties of T2 "ghost", it was found that the ratio of $S5/S7$ was 1.54, whereas the same ratio for whole phage was 1.43. This again suggests that the long head form is more permeable to solvent since removing the DNA would tend to enhance this difference. The direction of the change in $S5/S7$ indicates that the difference in solvent flow would decrease the relative sedimentation rate of the long head form.

With regard to deformation, SCHACHMAN[24] has stated that the intramolecular organization of a particle could vary, without a decrease in molecular weight, causing a drastic change in the effective volume but little change in the dimensional volume. That such a mechanism is operating in T2 is evidenced by the fact that the head dimensions of this particle do indeed change, without any change in the molecular weight. Thus it is seen that the apparent discrepancy between the effective volume and dimensional volume ratios can be accounted for by the anomalous behavior of the voluminous head of this virus. Furthermore, all these considerations support the conclusion that the sedimentation and diffusion properties of bacteriophage T2 are directly related to configurational changes occurring in the head protein.

ACKNOWLEDGEMENTS

This investigation was aided by grants from the National Foundation and Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago. One of us (L.M.K.) is a Senior Research Fellow of the U.S. Public Health Service (SF-185).

REFERENCES

1 A. E. HOOK, D. BEARD, A. R. TAYLOR, D. G. SHARP AND J. W. BEARD, *J. Biol. Chem.*, 165 (1946) 241.
2 D. G. SHARP, A. E. HOOK, A. R. TAYLOR, D. BEARD AND J. W. BEARD, *J. Biol. Chem.*, 165 (1946) 259.
3 I. J. BENDET, L. G. SWABY AND M. A. LAUFFER, *Biochim. Biophys. Acta*, 25 (1957) 252.
4 F. W. PUTNAM, *J. Biol. Chem.*, 190 (1951) 61.
5 N. W. TAYLOR, H. T. EPSTEIN AND M. A. LAUFFER, *J. Am. Chem. Soc.*, 77 (1955) 1270.
6 I. J. BENDET, J. L. ALLISON AND M. A. LAUFFER, *Virology*, 6 (1958) 571.
7 M. H. ADAMS, *Methods in Medical Research II*, Yearbook Publishers, Inc., Chicago, 1948, p. 1.
8 R. M. HERRIOTT AND J. L. BARLOW, *J. Gen. Physiol.*, 40 (1957) 809.
9 S. S. COHEN AND T. F. ANDERSON, *J. Exptl. Med.*, 84 (1946) 511.
10 M. SCHLESINGER, *Biochem. Z.*, 273 (1934) 306.
11 L. M. KOZLOFF AND F. W. PUTNAM, *J. Biol. Chem.*, 181 (1949) 207.
12 A. R. TAYLOR, *J. Biol. Chem.*, 165 (1946) 271.
13 R. M. HERRIOTT AND J. L. BARLOW, *J. Gen. Physiol.*, 36 (1952) 17.
14 H. K. SCHACHMAN, *Methods in Enzymology IV*, Academic Press, New York 1957, p. 32.
15 S. BRENNER AND R. W. HORNE, *Biochim. Biophys. Acta*, 34 (1959) 103.
16 R. C. WILLIAMS AND D. FRASER, *J. Bacteriol.*, 66 (1953) 458.

[17] E. KELLENBERGER AND W. ARBER, *Z. Naturforsch.*, 10b (1955) 698.
[18] S. M. LESLEY, R. C. FRENCH AND A. F. GRAHAM, *Can. J. Research, E* 28 (1950) 281.
[19] S. J. SINGER AND A. SIEGAL, *Science*, 112 (1950) 107.
[20] J. L. ONCLEY, *Ann. N.Y. Acad. Sci.*, 41 (1941) 121.
[21] H. A. SCHERAGA AND L. MANDELKERN, *J. Am. Chem. Soc.*, 75 (1953) 179.
[22] R. GANS, *Ann. Physik*, 86 (1928) 628.
[23] T. F. ANDERSON, C. RAPPAPORT AND N. A. MUSCATINE, *Ann. Institute Pasteur*, 84 (1953) 5.
[24] H. K. SCHACHMAN, *Ultracentrifugation in Biochemistry*, Academic Press, New York, 1959, p. 215.

Biochim. Biophys. Acta, 44 (1960) 445–458

PATTERNS OF CELLULAR CONTROLS OPERATING IN BACTERIOPHAGE REPRODUCTION

I. EFFECT OF 5-FLUOROURACIL ON THE MULTIPLICATION OF SEVERAL COLIPHAGES

FRED GOODMAN, JUSSI J. SAUKKONEN* AND ERWIN CHARGAFF
Cell Chemistry Laboratory, Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York, N.Y. (U.S.A.)

(Received February 24th, 1960)

SUMMARY

The effects of 5-fluorouracil on bacteriophage production in *E. coli*, strain B, have been studied. In the presence of the fluoropyrimidine the phage yields are less than 0.5 % of the control in T2r infection, whereas as much as 8 % of the control yield is obtained in T3 infection. The addition of uracil does not affect the inhibition of T2r multiplication, but restores T3 formation to 40–50 % of normal. Supplementation with thymidine has little effect on T3, but raises the yield of T2r to more than one fifth of the control. Essentially complete restoration of phage reproduction is observed when both uracil and thymidine are present together with fluorouracil.

Orienting experiments with phages T1 and T4r are also described.

INTRODUCTION

The nature of the control mechanisms that guarantee the unaltered reproduction of the various high polymers of the cell is among the most important problems in chemical biology[1]. There are many ways in which the study of this question may be undertaken, just as there must exist many such cellular controls; but it is clear that no direct approach to their description is discernible at present. We have, in the recent past, repeatedly discussed the conceptual basis of the problem[1–3] and attempted to define it through experimental studies[4–6]. Of particular interest, in this connection,

* Present address: State Serum Institute, Helsinki, Finland.